HAEMOGLOBIN ADDUCTS OF AROMATIC AMINES IN PEOPLE EXPOSED TO CIGARETTE SMOKE

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In a population-based study in Turin, Italy, smokers of blond tobacco showed 4-aminobiphenyl (4-ABP) adduct levels some three times higher than nonsmoking subjects, and smokers of black tobacco showed levels about five times greater than nonsmokers. A dose-response relationship between the number of cigarettes smoked per day and 4-ABP adduct level was observed, but did not account for the higher adduct levels observed in smokers of black tobacco. Smoking-related increases in haemoglobin adducts were also observed for *o*-toluidine, *p*-toluidine, 2,4-dimethylaniline and 2-ethylaniline. Smoking subjects showed 3-aminobiphenyl adduct levels about 12 times greater than those of nonsmokers, who rarely showed a detectable level. This may indicate that there are fewer sources of 3-aminobiphenyl exposure not related to tobacco smoke. Smokers of black tobacco showed higher adduct levels than smokers of blond tobacco for 4-ABP, *p*-toluidine and 2,4-dimethylaniline.

Epidemiological studies have shown an increased relative risk for urinary bladder cancer among cigarette smokers as compared to suitable nonsmoking control populations. These relative risks range from 1.5 to 3.0 (Vineis *et al.*, 1984). A recent Danish study has also implicated other forms of tobacco consumption (cigars/cigarillos, chewing tobacco and pipe tobacco) as factors for increased risk for bladder cancer (Mommsen & Aagard, 1983).

The work of Patrianakos and Hoffmann (1979) showed that the level of 4-ABP in the mainstream smoke of a US nonfilter cigarette, consisting of blond tobacco, was about 2.4 ng per cigarette. The level in the mainstream smoke of a French cigarette, containing black tobacco, was 4.6 ng. The work of Vineis *et al.* (1984) has shown that the risk for bladder cancer among smokers of black tobacco is higher than that of smokers of blond tobacco cigarettes. It is possible that the increased level of aromatic amines in the black tobacco cigarettes plays a role in the increased risk for bladder cancer in this population.

In order to determine whether the level of 4-ABP-haemoglobin adducts was greater in the blood of smokers of black tobacco, who were likely to have greater exposure to 4-ABP than smokers of blond tobacco, a study was conducted on the population of Turin, which was the site of the earlier epidemiological study (Vineis *et al.*, 1984).

Methods

Male volunteers were recruited at a blood donor centre to donate an additional 10-ml blood sample and to fill out a questionnaire on their smoking history and other

parameters, such as occupation. During two collection periods, 87 samples were collected, from 25 nonsmoking controls, 40 smokers of blond tobacco, 18 smokers of black tobacco, three smokers of mixed blond/black tobacco and one cigar smoker. The samples were recoded so that the identity of the samples would be unknown at the time of the analysis. The washed red blood cells were sent to the USA in a coded, blind fashion for analysis of the adduct levels.

The method of analysis of 4-ABP has been described (Bryant *et al.*, 1987) and involves gas chromatography-mass spectrometry. Analysis for other amines was achieved by scanning chromatograms at suitable single ions. Additional internal standards included d_5-aniline and 5-F-2-naphthylamine.

Results

4-Aminobiphenyl

Comparison of the 4-ABP-haemoglobin adduct levels in the three groups (nonsmokers, smokers of blond and of black tobacco) shows that both smoking groups have elevated adduct levels compared to nonsmokers, and that smokers of black tobacco have somewhat higher adduct levels than smokers of blond tobacco. The difference between these three groups was found to be statistically significant ($p = 0.0001$) by analysis of variance. The unadjusted means and standard errors of the 4-ABP-haemoglobin adduct levels are 51 ± 18 for nonsmokers, 176 ± 14 for smokers of blond tobacco and 288 ± 21 for smokers of black tobacco.

The age distribution by type of tobacco showed that a higher proportion of older subjects smoked black tobacco (90% were over 35, compared with 50% of smokers of blond tobacco). In addition, the smokers of black tobacco consumed more cigarettes per day than the smokers of blond tobacco (black: 67% smoked 20 + cigarettes/day; blond: 44% smoked 20 + cigarettes/day). The data were thus analysed as to whether 4-ABP adduct levels were correlated with age or amount smoked per day. The age of the subjects showed a trend that was correlated with higher adduct levels but was not statistically significant ($p = 0.099$). The amount smoked per day was highly correlated ($p = 0.0015$) with adduct level. The dose-response relationship was examined within each tobacco type and found to be statistically significant for blond tobacco ($p = 0.0074$). A trend among the smokers of black tobacco was evident, but was not statistically significant ($p = 0.183$). These dose-response relationships are given in Table 1. Within each category of amount smoked, the smokers of black tobacco showed adduct levels that were 40–50% higher than those of smokers of blond tobacco.

Table 1. 4-ABP-haemoglobin adduct levels by amount smoked (least squares means and standard errors)

Amount smoked (no. of cigarettes/day)	Blond tobacco	Black tobacco
<10	124.4 (24.9)	—
10 – 19	154.5 (19.3)	222.7 (57.8)
20 +	216.4 (17.1)	321.2 (40.8)

Table 2. Adduct levels observed (least squares means and standard errors)[a]

Aromatic amine	Nonsmokers (25)		Tobacco type			
			Blond (43)		Black (18)	
2-Naphthylamine	11.6	(1.1)	17.2	(1.8)	21.3	(4.9)
o-Toluidine	188	(19)	290	(19)	329	(22)
m-Toluidine	1141	(138)	1097	(108)	1140	(138)
p-Toluidine	209	(24)	306	(35)	415	(73)
2-Ethylaniline	38	(3)	70	(10)	80	(12)
3-Ethylaniline	102	(16)	115	(22)	129	(34)
2,5-Dimethylaniline	50	(6)	67	(10)	70	(14)
2,4-Dimethylaniline	40	(5)	73	(10)	114	(17)
2,6-Dimethylaniline	264	(90)	86	(15)	98	(30)
2,3-Dimethylaniline	52	(8)	56	(11)	64	(19)
3,5-Dimethylaniline	93	(12)	112	(31)	135	(31)
3,4-Dimethylaniline	47	(11)	46	(11)	65	(19)
3-Aminobiphenyl	1.2	(0.4)	13.8	(1.9)	12.9	(3.0)
4-Aminobiphenyl	51	(18)	176	(14)	288	(21)

[a] Values expressed in pg amine/g haemoglobin

Substituted anilines, 2-naphthylamine and 3-aminobiphenyl

The adduct levels for the remaining amines observed in the three groups are given in Table 2. Methods for determination of adducts with other aromatic amines may not have the same degree of accuracy as those for 4-ABP and 3-aminobiphenyl. Thus, the levels were examined in a semiquantitative manner to determine if they correlated with smoking status, or perhaps with type of tobacco. Adducts with seven amines appeared to be associated with smoking status: 4-ABP, 3-aminobiphenyl, 2-naphthylamine, *o*-toluidine, *p*-toluidine, 2-ethylaniline and 2,4-dimethylaniline. Only five of these amines, however, showed an association with tobacco type: 4-ABP, *o*-toluidine, *p*-toluidine, 2-ethylaniline and 2,4-dimethylaniline. In the case of 3-aminobiphenyl, a dose-response relationship was observed among smokers of blond tobacco ($p = 0.02$), but not for smokers of black tobacco. Thus, for smokers of both tobacco types, there was a dose-response relationship of borderline significance ($p = 0.06$).

Conclusions

Due to the complex nature of tobacco smoke, it may ultimately be difficult to prove that it is the aromatic amines contained in the smoke that are responsible for the increased bladder cancer risk in smoking populations. Other types of compounds present in the smoke may also be human bladder carcinogens: some *N*-nitroso compounds are bladder carcinogens in various species, and these may also be produced during tobacco smoking. *N*-Nitrosodi-*n*-butylamine is a bladder carcinogen in rats, mice, hamsters and guinea-pigs (IARC, 1974) and has been detected in tobacco smoke (Schmeltz & Hoffmann, 1977). Nonetheless, it is the aromatic amines which appear most likely to be the cause of the increased risk for bladder cancer among cigarette smokers.

It is interesting to note that the adduct ratios for smokers of black and blond tobacco *versus* nonsmokers closely resemble the relative risks for bladder cancer seen historically in these populations (Vineis *et al.*, 1984). One conclusion to be drawn from this finding is that the aromatic amines, including 4-ABP, are involved not only in the

increased bladder cancer risk of cigarette smokers but also in the increased potency of black tobacco. Thus, the adduct levels of 4-ABP would be two to three times higher in the blood of smokers of blond tobacco and five to six times higher in the blood of smokers of black tobacco as compared to nonsmokers. The bladder cancer risk of any individual is a function of a great number of factors: the amount and duration of smoking, type of tobacco, exposure to promoting agents, interaction of occupation, and biochemical parameters such as urine pH and metabolic status (e.g., acetylator phenotype). Nonetheless, when comparing groups of individuals, among whom differences may be normally distributed or may offset one another, the overall risk for the group may be correlated with one of these factors. Although the exact relationship between 4-ABP-haemoglobin adduct level and bladder cancer risk is not understood, it appears that measurement of this parameter may be a useful step in examining the biochemical basis for the increased risk in smokers of blond and black tobacco.

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